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Insights into the Essential Oil Compositions of Brazilian Red and Taiwanese Green Propolis

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The objective of the present study was to characterize chemically the essential oils of two distinct propolis types: Brazilian red and Taiwanese green. Unlike the non-volatile chemical composition of these types of propolis, which has been extensively studied, the knowledge of the essential oils is scarce or even not investigated. The essential oils were obtained by hydrodistillation of raw propolis samples using a Likens-Nickerson type apparatus and then analyzed by GC/MS. The main volatile components of Brazilian red propolis were the phenylpropanoids: elemicin (26.1-27.5%), methyl eugenol (16.3-23.8%), *trans*-methyl isoeugenol (9.2-11.6%), isoelemicin (6.1-7.1%) and *trans*-anethole (4.4-7.1%), while the major constituents of Taiwanese green propolis essential oil were: β-eudesmol (13.9%), 6-methyl-3,5-heptadiene-2-one (12.2%), γ-eudesmol (4.4%), geranial (4.1%) and 6-methyl-5-heptene-2-one (3.7%).

Keywords: Essential oil, Brazilian red propolis, Taiwanese green propolis, Phenylpropanoids, β-Eudesmol.

Propolis (bee glue) is a bee product of plant origin, which is used in the hive as building material and a defensive substance. It is a complex mixture of resin, collected by bees from plant buds and exudates, and beeswax. Since ancient times, propolis has been widely used in folk medicine. Nowadays, its beneficial properties and biological activities have been extensively studied and it is proved that propolis exhibits antimicrobial, antioxidant, anti-inflammatory, immunostimulating, hepatoprotective as well as cytotoxic activity as it contains a large number of bioactive molecules [1, 2]. The chemical composition of bee glue is highly variable and depends mainly on the local flora, but despite the differences in origin and composition, propolis samples show very similar biological activity [3]. This leads to the continued increase of propolis use, for example in OTCs (over-the-counter preparations), "bio"-cosmetics and functional foods. Raw propolis is composed of about 50% resin and vegetable balsam (consisting mainly of phenolics and di- and triterpenoids), about 30% wax, 10% essential and aromatic oils, 5% pollen, and other substances [4]. In spite of their low concentrations volatile compounds of propolis are of importance because they are known to possess valuable biological activities, especially antibacterial, which contribute to the biological activity of propolis. This fact, along with their pleasant aroma, makes them of high relevance for cosmetics and especially for the food industry as preservatives [5, 6]. Therefore, the present work focuses on the determination of the essential oil chemical compositions of two distinct propolis types: Brazilian red and Taiwanese green. Unlike the non-volatile chemical composition of these types of propolis, which has been extensively studied, their essential oils are little or not investigated. There are only data on the chemical composition of volatiles obtained by a dynamic headspace technique from Brazilian red propolis [7]. Till now the chemical composition and biological activity of Taiwanese green propolis essential oil have never been investigated.

The essential oils of Brazilian red (three samples) and Taiwanese green (one sample) propolis were obtained by hydrodistillation of raw propolis samples in a Likens-Nickerson type apparatus and then analyzed by GC/MS. In Brazilian red propolis essential oil (samples R-1, R-2 and R-3), for which the main plant source is *Dalbergia ecastophyllum*, 69 volatiles were identified, which constitute 92.1%, 97.8% and 96.4% of the oil, respectively. The data obtained are summarized in Table 1. The quantities measured correspond to the percentage of total ion current and are not a true quantitation. However, they are completely reliable in the case of comparison between samples having similar qualitative composition [8, 9].

In all three studied samples the main volatile components, comprising significant parts of the total oil, were the phenylpropanoids: elemicin (26.1-27.5%), methyl eugenol (16.3-23.8%), *trans*-methyl isoeugenol (9.2-11.6%), isoelemicin (6.1-7.1%) and *trans*-anethole (4.4-7.1%), a mixture which we have already isolated from Brazilian red propolis [10]. Partially our results corroborate those of Nunes *et al*., who investigated the volatile fraction of Brazilian red propolis, obtained by a dynamic headspace technique, and also found phenylpropanoids as the major constituents [7]. However, as expected, the differences were both quantitative and qualitative, which once again proves that the chemical composition of an essential oil is dependent on the extraction method [11]. The presence of high amounts of phenyl propanoids in Brazilian red propolis essential oil seems to be unique comparing it with other types of propolis volatiles [12]. As regards the other components of the oil, in all three samples, as in poplar type propolis essential oil, sesquiterpenes predominate over monoterpenes. Monoterpene hydrocarbons are missing and oxygenated monoterpenes, except for linalool, are contained only in sample R-3. β-Caryophyllene and spathulenol (R-1), α-copaene (R-2 and R-3) and β-bisabolene (R-3) were also in appreciable percentages.

Taiwanese green propolis is a Pacific type and its plant source is *Macaranga tanarius*. Till now its essential oil composition is unreported. Hydrodistillation of raw propolis produced a colorless volatile oil, which was analyzed by GC/MS. The chemical composition of the essential oil is summarized in Table 2.

Among 91 identified constituents, representing approximately 77% of the total oil, β-eudesmol (13.9%), 6-methyl-3,5-heptadiene-2-one (12.2%) and γ-eudesmol (4.4%) were the most abundant.

Table 1: Chemical composition of Brazilian red propolis essential oil

No	Compounds	RI[a]	RI[b]	% of TIC[c]		
				R-1	R-2	R-3
	Oxygenated monoterpenes			**0.1**	**0.2**	**2.1**
1	Linalool	**1 101**	1 101	0.1	0.2	0.1
2	*p*-Mentha-1,5-dien-8-ol	**1 167**	1 166	-	-	0.2
3	Terpinen-4-ol	**1 186**	1 187	-	-	0.4
4	*p*-Cymen-8-ol	**1 191**	1 193	-	-	0.5
5	α-Terpineol	**1 199**	1 198	-	-	0.4
6	Eucarvone	**1 216**	1 223	-	-	0.3
7	Thymol	**1 296**	1 296	-	-	0.1
8	Carvacrol	**1 303**	1 304	-	-	0.1
	Sesquiterpene hydrocarbons			**9.0**	**6.7**	**12.7**
9	α-Cubebene	**1 352**	1 351	0.4	0.4	0.2
10	α-Copaene	**1 383**	1 382	1.5	2.5	3.5
11	β-Bourbonene	**1 391**	1 391	0.5	-	-
12	α- Gurjunene	**1 416**	1 412	-	-	0.1
13	α-*cis*-Bergamotene	**1 418**	1 420	0.3	0.7	1.3
14	β-Caryophyllene	**1 429**	1 428	2.6	0.5	0.9
15	α-*trans*-Bergamotene	**1 438**	1 438	0.3	0.2	0.2
16	α-Caryophyllene	**1 466**	1 465	0.6	0.1	0.2
17	*cis*-Muurola-4(14),5-diene	**1 472**	1 470	0.2	tr[d]	0.1
18	*trans*-Cadina-1(6),4-diene	**1 480**	1 477	tr	0.1	0.2
19	γ-Muurolene	**1 483**	1 485	0.3	0.1	0.2
20	α-Curcumene	**1 485**	1 486	tr	0.1	0.3
21	*trans*-Muurola-4(14),5-diene	**1 491**	1 491	0.3	-	-
22	β-Bisabolene	**1 513**	1 513	0.4	0.9	2.0
23	γ-Cadinene	**1 522**	1 514	0.2	tr	0.1
24	δ-Cadinene	**1 525**	1 525	0.9	0.7	1.5
25	*trans*-Calamenene	**1 530**	1 529	0.3	0.3	1.7
26	*trans*-Cadina-1(2),4-diene	**1 541**	1 537	0.2	0.1	0.2
27	β-Calacorene	**1 572**	1 573	tr	-	tr
	Oxygenated sesquiterpenes			**5.6**	**2.7**	**2.9**
28	E-Nerolidol	**1 564**	1 565	0.3	tr	0.1
29	Spathulenol	**1 589**	1 590	2.2	0.5	0.6
30	Caryophyllene oxide	**1 595**	1 593	1.3	0.3	0.2
31	Salvial-4(14)-en-1-one	**1 605**	1 603	0.1	-	-
32	*epi*-Cedrol	**1 626**	1 632	0.1	0.4	0.5
33	1-*epi*-Cubenol	**1 639**	1 629	0.4	0.3	0.7
34	*epi*-α-Cadinol	**1 653**	1 651	0.2	0.1	0.7
35	α-Cadinol	**1 666**	1 666	0.7	tr	0.1
36	α-Eudesmol	**1 669**	1 654	-	1.1	-
37	Eudesma-4(15),7-diene-1-β-ol	**1 703**	1 690	0.3	-	-
	Phenylpropanoids			**68.0**	**82.6**	**71.2**
38	Isoanethole	**1 202**	1 201	0.1	0.2	0.2
39	*trans*-Anethole	**1 291**	1 290	4.5	7.1	4.4
40	Eugenol	**1 358**	1 358	0.3	0.3	0.4
41	Methyl eugenol	**1 403**	1 402	16.3	23.8	20.4
42	*trans*-Isoeugenol	**1 454**	1 453	0.6	0.9	0.6
43	*trans*-Methyl isoeugenol	**1 499**	1 500	9.4	11.6	9.2
44	Elemicin	**1 550**	1 552	26.1	27.5	27.3
45	Methoxyeugenol	**1 600**	1 609	3.6	4.4	2.6
46	Isoelemicin	**1 649**	1 649	7.1	6.8	6.1
	Acids, esters, amides			**3.4**	**2.0**	**1.7**
47	Caproic acid	**988**	984	0.4	-	0.1
48	Octanoic acid	**1 175**	1 176	0.4	-	0.3
49	Nonanoic acid	**1 270**	1 271	0.2	-	-
50	*trans*-Cinnamyl acetate	**1 450**	1 452	0.2	0.2	0.3
51	Benzyl benzoate	**1 779**	1 774	0.6	-	-
52	*cis*-Benzyl cinnamate	**1 961**	1 959	0.6	-	-
53	*trans*-Benzyl cinnamate	**2 115**	2 102	0.3	-	-
54	Oleamide	**2 365**	2 362	0.7	1.8	1.0
	Alcohols, aldehydes, ketones			**1.3**	**0.4**	**1.7**
55	Benzyl alcohol	**1 040**	1 039	0.2	-	-
56	*n*-Decanal	**1 208**	1 207	tr	0.1	-
57	*m*-Guaiacol	**1 232**	1 235	0.5	0.1	0.5
58	*p*-Anisaldehyde	**1 263**	1 258	0.1	-	-
59	*trans*-Cinnamaldehyde	**1 282**	1 272	0.5	0.2	1.2
	Aliphatic hydrocarbons			**4.7**	**3.2**	**4.1**
60	*n*-Dodecane	**1 199**	1 200	0.3	0.4	0.6
61	*n*-Tridecane	**1 300**	1 300	0.1	0.3	0.3
62	*n*-Heptadecane	**1 700**	1 700	0.4	0.6	0.5
63	*n*-Octadecane	**1 800**	1 800	0.1	0.2	0.2
64	*n*-Nonadecane	**1 900**	1 900	0.1	0.2	0.2
65	*n*-Heneicosane	**2 102**	2 100	0.6	0.3	0.5
66	*n*-Tricosane	**2 301**	2 300	0.9	0.4	0.7
67	*n*-Pentacosane	**2 501**	2 500	1.0	0.4	0.6
68	*n*-Heptacosane	**2 701**	2 700	1.0	0.4	0.5
69	*n*-Nonacosane	**2 901**	2 900	0.2	-	-

[a] Retention indices on apolar column (HP-5MS); [b] Retention indices on apolar column of literature ([15] and NIST); [c] The total ion current (TIC) generated depends on characteristics of the compound concerned and is not a true quantification; [d] Traces of compound identified. In quantification of different classes of compounds the amounts of traces are not included.

Table 2: Chemical composition of Taiwanese green propolis essential oil

No	Compounds	RI[a]	RI[b]	% of TIC[c]
	Monoterpene hydrocarbons			**0.2**
1	Carvomenthene	**1 023**	1 025	0.2
2	*p*-Cymenene	**1 093**	1 093	tr[d]
	Oxygenated monoterpenes			**12.2**
3	Bergamal	**1 056**	1 057	0.4
4	*trans*-Dihydrorose oxide	**1 074**	1 073	1.7
5	*cis*-Vertocitral C	**1 080**	1 080	0.1
6	*cis*-Linalool oxide	**1 089**	1 087	tr
7	Linalool	**1 102**	1 101	0.5
8	*cis*-*p*-Mentha-2,8-dien-1-ol	**1 141**	1 138	0.1
9	Borneol	**1 178**	1 177	0.2
10	*p*-Cymen-8-ol	**1 191**	1 183	0.5
11	γ-Terpineol	**1 199**	1 199	0.6
12	*iso*-Dihydro carveol	**1 208**	1 215	0.1
13	Verbenone	**1 214**	1 205	0.4
14	Neral	**1 242**	1 238	1.6
15	Geraniol	**1 252**	1 253	0.3
16	Piperitone	**1 260**	1 253	0.1
17	Geranial	**1 271**	1 267	4.1
18	Thymol	**1 295**	1 290	0.2
19	Piperitenone	**1 347**	1 343	0.1
20	Ethyl-Z-geraniate	**1 351**	1 354	0.2
21	*trans*-Geranyl acetone	**1 450**	1 451	1.0
	Sesquiterpene hydrocarbons			**2.3**
22	α-Copaene	**1 383**	1 377	0.2
23	9-*epi*-*trans*-Caryophyllene	**1 470**	1 467	tr
24	β-Chamigrene	**1 481**	1 482	0.3
25	α-Curcumene	**1 486**	1 486	tr
26	Viridiflorene	**1 499**	1 497	tr
27	*trans*-γ-Cadinene	**1 523**	1 514	tr
28	δ-Cadinene	**1 526**	1 528	0.1
29	*trans*-Calamenene	**1 530**	1 529	0.2
30	α-Calacorene	**1 552**	1 550	0.1
31	β-Calacorene	**1 572**	1 574	tr
32	Cadalene	**1 684**	1 684	1.4
	Oxygenated sesquiterpenes			**20.9**
33	β-Agarofuran	**1 516**	1 516	0.3
34	Italicene ether	**1 535**	1 538	tr
35	Ledol	**1 584**	1 588	0.1
36	Caryolan-8-ol	**1 589**	1 573	0.3
37	Gleenol	**1 595**	1 595	tr
38	10-*epi*-γ-Eudesmol	**1 630**	1 624	0.2
39	Citronellyl pentanoate	**1 630**	1 625	0.2
40	1-*epi*-Cubenol	**1 639**	1 629	0.2
41	γ-Eudesmol	**1 643**	1 632	4.4
42	Hinesol	**1 652**	1 642	1.0
43	*epi*-α-Muurolol	**1 655**	1 654	0.1
44	δ-Cadinol	**1 657**	1 646	0.1
45	α-Cadinol	**1 667**	1 654	0.1
46	β-Eudesmol	**1 669**	1 651	13.9
	Phenylpropanoids			**0.5**
47	*E*-Anethole	**1 292**	1 285	tr
48	*trans*-Isoeugenol	**1 455**	1 453	0.1
49	*trans*-Methyl isoeugenol	**1 498**	1 499	tr
50	Elemicin	**1 549**	1 552	0.3
51	*cis*-Isoeugenol acetate	**1 567**	1 568	0.1
	Alcohols, aldehydes, ketones			**19.7**
52	2-Methylcyclopentanone	**845**	843	0.1
53	6-Methyl-5-hepten-2-one	**987**	986	3.7
54	6-Methyl-5-hepten-2-ol	**995**	995	0.1

No	Compounds	RI[a]	RI[b]	% of TIC[c]
55	Benzyl alcohol	**1 040**	1 039	0.2
56	o-Guaiacol	**1 091**	1 092	0.6
57	6-Methyl-3,5-heptadiene-2-one	**1 107**	1 107	12.2
58	Phenyl ethyl alcohol	**1 118**	1 117	0.1
59	*E*-Cinnamaldehyde	**1 282**	1 270	0.2
60	2-Heptadecanone	**1 902**	1 900	1.9
61	Octadecanol	**2 089**	2 078	0.1
62	2-Nonadecanone	**2 107**	2 101	0.4
63	2-Heneicosanone	**2 311**	2 309	0.1
	Acids, esters			**3.1**
64	4-Methylpent-2-enolide	**952**	952	0.1
65	Caproic acid	**991**	984	0.3
66	Heptanoic acid	**1 083**	1 080	0.1
67	Octanoic acid	**1 176**	1 176	0.2
68	Pelargonic acid	**1 274**	1 271	tr
69	*o*-Anisic acid methyl ester	**1 337**	1 336	0.9
70	*trans*-Methyl cinnamate	**1 393**	1 392	tr
71	Phenyl ethyl 2-methylbutanoate	**1 490**	1 487	tr
72	*n*-Decanoic acid	**1 367**	1 367	0.5
73	Benzyl benzoate	**1 779**	1 760	0.3
74	Benzyl salicylate	**1 883**	1 866	0.7
	Aliphatic hydrocarbons			**17.7**
75	Dodecane	**1 200**	1 200	0.6
76	Tridecane	**1 300**	1 300	0.7
77	Tetradecane	**1 400**	1 400	1.6
78	Pentadecane	**1 500**	1 500	1.6
79	Hexadecane	**1 600**	1 600	1.0
80	Heptadecane	**1 699**	1 700	1.5
81	Octadecane	**1 800**	1 800	0.6
82	Eicosane	**1 999**	2 000	0.1
83	Heneicosane	**2 100**	2 100	0.5
84	Docosane	**2 201**	2 200	0.1
85	Tricosane	**2 301**	2 300	3.5
86	Tetracosane	**2 401**	2 400	0.1
87	Pentacosane	**2 501**	2 500	2.4
88	Hexacosane	**2 601**	2 600	0.1
89	Heptacosane	**2 701**	2 700	1.2
90	Squalene	**2 816**	2 790	1.9
91	Nonacosane	**2 901**	2 900	0.2

[a] Retention indices on apolar column (HP-5MS); [b] Retention indices on apolar column of literature ([15] and NIST); [c] The total ion current (TIC) generated depends on characteristics of the compound concerned and is not a true quantification; [d] Traces of compound identified. In quantification of different classes of compounds the amounts of traces are not included.

Oxygenated mono- and sesquiterpenes predominate over terpene hydrocarbons and sesquiterpenes are more than monoterpenes. The oxygenated monoterpenes represent 12.2% of the oil, with geranial as the main part. Unlike Brazilian red propolis volatiles, the Taiwanese green propolis essential oil contains a significant amount of aliphatic alcohols, aldehydes and ketones (19.7%) and very low amounts of phenylpropanoids (0.5%). The amount of aliphatic hydrocarbons is significant (17.7% of the total oil), with tricosane the principal compound. The major compound, β-eudesmol, is very typical for poplar type propolis volatiles. The main components of popular Brazilian green propolis essential oil, β-caryophyllene and *trans*-nerolidol, are missing [13, 14]. An appreciable part of the compounds remained unidentified, so further research is needed for their identification.

The present work provides additional insights into Brazilian red and Taiwanese green propolis concerning the chemical composition of their volatiles. The phenylpropanoids, identified as main components of Brazilian red propolis essential oil, can be eventually used as its chemical markers. As far as it concerns Taiwanese green propolis this is the first report about the chemical composition of its essential oil. However, further research is needed to reveal the biological activity of the investigated volatiles, as well as their potential and contribution to the biological activity and medicinal application of propolis.

Experimental

Propolis samples: The investigated propolis samples are commercial. Their plant sources and propolis type are determined by TLC comparison with authentic samples.

Essential oil extraction: The raw propolis samples were grated after cooling and subjected to hydrodistillation in a Likens-Nickerson type apparatus for 4 h. The collected distillates were extracted with diethyl ether/*n*-pentane (1:1). The extracts obtained were dried over anhydrous Na_2SO_4, evaporated and stored in a freezer until GC/MS analysis. The essential oil content of the Brazilian red propolis samples was 0.12% (R-1) and 0.11% (R-2, R-3) and of the Taiwanese green- 0.05% based on the weight of raw propolis.

GC/MS analysis: Analysis of the oils was performed on a Hewlett-Packard gas chromatograph 5890 series II Plus linked to a Hewlett–Packard 5972 mass spectrometer system equipped with a HP5-MS capillary column (30 m x 0.25 mm and 0.25 μm film thickness). The ion source was set at 250°C and the ionisation voltage at 70 eV. The temperature was programmed from 40 – 280°C at a rate of 6°C/min, and helium was used as the carrier gas at a flow rate of 0.8 mL/min. The split ratio was 1:10, the injector temperature 280°C. Acquisition mass range m/z 35–750; scan time 49 min. The identification of the compounds was based on their retention indices (RI), obtained using *n*-alkanes (C8–C40), and by comparison of their EI-mass spectra with NIST library spectra and literature [15].

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